DYNAMICS OF YELLOW STARTHISTLE, *CENTAUREA SOLSTITIALIS*, ACHENES UNDER BURIED, STORED, AND NATURAL CONDITIONS IN CALIFORNIA

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ABSTRACT

Studies were conducted to compare changes in germinability and/or viability of yellow starthistle achenes buried (in packets) in soil or stored dry in the laboratory, and to determine the rate of achene depletion from a natural soil seed bank, In one study, after 72 months there were 0% to 96% germinable achenes remaining in packets after burial, and a mean above 99% after dry storage. In another study, total live achenes (germinable plus viable) declined from 100% at harvest to 73.9% (pappus-bearing) and 81.3% after 24 months of burial for pappus-bearing and nonpappus-bearing achenes, respectively. Germination percentages of pappus-bearing achenes, buried at various depths, increased with depth. Maximum survival of achenes occurred at depths of 5-cm or more, after 12 months of burial. The density of yellow starthistle achenes and seedlings declined curvilinearly in a natural soil seed bank when achene rain was prevented. Decline was attributed primarily to seedling emergence and achene death. Achene density in the upper 2.5-cm of soil appeared to be a good predictor of seedling emergence after autumn rains.

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INTRODUCTION

Yellow starthistle (Centaurea solstitialis L. # CENSO), a weed of Eurasian origin, has become widespread and particularly abundant in California, Oregon, Washington and Idaho (7). In California alone, this weed infests more than 3 million gross ha (7), much of it concentrated in the north coast and Sacramento drainage areas (6). Yellow starthistle is especially troublesome in rangelands due to its potential to form dense stands and reduce forage capacity and interfere with grazing (2).

Yellow starthistle is normally a winter annual (2, 6, 7). The life cycle of most plants begins with seedling emergence in autumn and ends with flowering and achene production during the following summer. Two kinds of achenes are produced, a light-colored type bearing divergent pappus bristles ("pappus-bearing"), and a dark-colored type, usually without pappus bristles ("nonpappus-bearing") (5).

Control of annual weeds, such as yellow starthistle, depends on consistent suppression of achene production and depletion of the soil seed bank. Despite the importance of yellow starthistle as a weed, little has been reported about its biology, including the fate of the achenes in soil. To enhance the evaluation of biological control agent impact on yellow starthistle, the California Department of Food and Agriculture, Biological Control Program, in collaboration with the USDA Biological Control of Weeds Laboratory (Albany, California), initiated studies on seed longevity. Since yellow starthistle is a winter annual, relying on seed production to perpetuate itself, and because most of the candidate biological control agents impact the flower heads of their host, it is important to have basic information on seed dynamics. In the present research, studies were carried out to compare the changes in germinability or viability of the two achene types buried in soil or held in dry storage and to determine the rate at which the achenes are depleted from a natural soil seed bank.

MATERIALS AND METHODS

Experimental sites. All field studies were carried out near Loomis, Placer County, California. The site had rolling terrain at elevations of 110- to 150 m. Formerly orchard land, it had lain fallow for many years and had become infested with yellow starthistle mixed with various grasses, forbs and legumes. The soil, classified as an Andregg sandy-loam (coarse-loamy, mixed thermic Typic Haploxerolls) (1), is well drained except in low-lying areas.

Germination conditions. All achene germination tests were carried out at the California Department of Food and Agriculture Seed Analysis Laboratory in Sacramento. Non-deteriorated achenes were placed on two sheets of blue-blotting germination paper in plastic trays (25- by 17- by 3-cm). The germination paper was moistened with tap water and the achenes were incubated for 21 days in automatic germinators set at an alternating temperature and light regime of 25°C (8 hrs cool white fluorescent light, 60 umols.$m^{-2} \cdot s^{-1}$, at achene level) and 15°C (16 hrs darkness) at >95% relative humidity. Germinated achenes were counted and discarded at weekly intervals. Protrusion of the radicle through the testa was the criterion of germination. Where noted, the remaining firm, ungerminated achenes were checked for viability by slicing each in half lengthwise (narrow axis) and noting the condition of the endosperm using a stereo microscope (30X). Those with a firm white endosperm were counted as viable. Data is presented either as percentages of germinated or total live (germinated plus viable) achenes that were buried or placed in dry storage (burial studies) or as mean numbers of total live achenes m^{-2} recovered from soil cores (depletion study).

Achenes buried at 5-cm or stored dry.

1983 study. Four replicate samples of pappus-bearing and nonpappus-bearing achenes (3000 each) were collected in August 1983. The pappus-bearing achenes were vacuumed from open heads; the nonpappus-bearing achenes and chaff were separated from the heads by crushing and screening (2.0 mm mesh). Empty testa were eliminated by air-stratification with a seed blower. Processed achenes were separated by type and replicate field sample.

Four replicate lots of each achene type (100 achenes per lot) were drawn (one replicate lot per field-collected sample) per burial time interval and mixed with 12 ml of steam-sterilized sand, enclosed in 5-cm square nylon mesh packets and buried in soil to a depth of 5-cm in a randomized block design. A duplicate set of lots were drawn and enclosed in vapor-tight glass vials and stored in darkness in the laboratory at 21° ± 2°C. Dry-stored achenes were tested for germinability at 1 week, 1, 12, 36, 50, and 72 months post-harvest. Buried achenes were tested at 1 week, 1, 12, 24, 36, 50, and 72 months post-harvest.

1984 study. Pappus-bearing and nonpappus-bearing achenes were collected, processed, and stored as in 1983. Before achenes were drawn for germination, burial, or dry storage, the four replicate field samples were pooled, and ten replicate lots of each achene type (80 achenes per lot) were drawn per burial and storage time interval. Dry-stored achenes were tested for germinability at 1 week, 1 and 24 months post-harvest. Buried achenes were tested at 1 week, 1, 12, and 24 months post-harvest. Only nine replications were exhumed at 24 months. The remaining firm, ungerminated achenes were checked for viability as described previously.

Pappus-bearing achenes buried at various depths.

1984 study. Eight replicate lots (per burial depth) of pappus-bearing achenes (80 achenes per lot) were enclosed (without sand) in nylon mesh packets and buried in soil to a depth of 0.5-, 1-, 2.5-, or 5-cm in October 1984. Achenes were drawn from the same source used in the previous 1984 study. The packets were arranged by burial depth in a randomized block design. Packets were retrieved in November 1985 and the achenes tested for germinability as described previously.

1988 study. Packets of pappus-bearing achenes, collected in August 1988, were buried to a depth of 0.5-, 1-, 1.5-, 2-, 2.5-, 5- or 10-cm in October 1988. The packets were retrieved in September 1989 and the number of firm, intact achenes and empty (split or un-split) testa counted. The intact achenes were tested for germinability, and the remaining firm, ungerminated achenes checked for viability as described previously.

Achene depletion. In a preliminary study, analysis of yellow starthistle aohene numbers in soil indicated that 30 quadrats with eight soil cores per quadrat (two cores each per quadrant) pooled would be an adequate sample size. At the depletion study site (15- by 15-m) four sets of 30 quadrats (0.5- by 0.5-m) each were randomly selected for obtaining soil cores, one set for each year sampled, from 1983 through 1986. Another set of 30 quadrats (0.25- by 0.25-m) was randomly selected and permanently marked for determining yellow starthistle seedling emergence. Galvanized nails were firmly anchored in soil at the corners of each of these quadrats, and braided nylon twine was affixed to the nails, slightly above ground level, to establish the perimeter.

During summer and autumn 1983, yellow starthistle plants growing within the study site were allowed to disseminate achenes. Thereafter, all further seed rain was prevented inside the site and around the periphery (three m-wide strip) by mowing or hand clipping plants.

Yellow starthistle seedlings in each of the 30 permanently marked (0.25- by 0.25-m) quadrats were counted during the rainy season (September through March) for four years beginning in 1983. Counts were initiated two to three weeks after the first measurable rainfall of the season and continued at monthly intervals. Newly emerged seedlings were marked with flagged wires to avoid duplicating counts.

Each year in September, from 1983 through 1986, a quadrat frame (0.5- by 0.5-m), divided into 100 equal cells, was positioned over each of 30 quadrats, and eight soil cores (1.9-cm diam by 2.5-cm depth plus litter) were extracted with a soil auger from randomly chosen cells and combined. Each of these composite soil samples was washed separately in a nylon mesh bag, and the non-deteriorated yellow starthistle achenes were removed with forceps, air-dried and tested for germinability and viability as described previously.

Statistical Analysis

Linear and non-linear regression analyses were performed on data using the general linear model and the nonlinear regression procedures (Marquardt method), respectively, of the Statistical Analysis System (8). Levene's test was used to assess the homogeneity of variance in the germinability (with angular transformation) (10) of burled achenes (9). Fisher's least significant difference test for pairwise means comparisons was used to evaluate germination percentages of dry-stored and buried achenes between time of collection and one month post-harvest.

RESULTS

Fate of achenes buried at **5-cm** or stored dry.

1983 study. Achenes, incubated one week after harvest, had a mean germination percentage of 84.3%. After 72 months, dry-stored and buried achenes had mean germination percentages of 99.4 and 37.7, respectively. The mean percentage of germinable achenes (with angular transformation) stored in the laboratory showed a significant curvilinear increase over 72 months (r^2 = .59, p = .001). Although there was a decline in mean germination percentages of buried achenes, regression analysis of achene germinability was inappropriate given the heterogeneity of variance between months. Regression analysis indicated that for buried achenes the variability in germination percentage (with angular transformation) showed a significant linear increase over 72 months (r^2 = .73, $p < .01$). The relationship between time and variability in germination percentage is given by:

$$V = 0.056 + 0.003M$$

where V = variance in germination percentage, and M = months of burial. Over time, achenes in some packets retained a high germination percentage, while in others achenes displayed a much lower germination percentage (Fig. 1).

1984 study. There was a decline in percentages of total live (germinable plus viable), buried pappus-bearing and nonpappus-bearing achenes over time. However, relatively large percentages remained alive at the end of the two year study. After 24 months, viability of buried pappus-bearing and

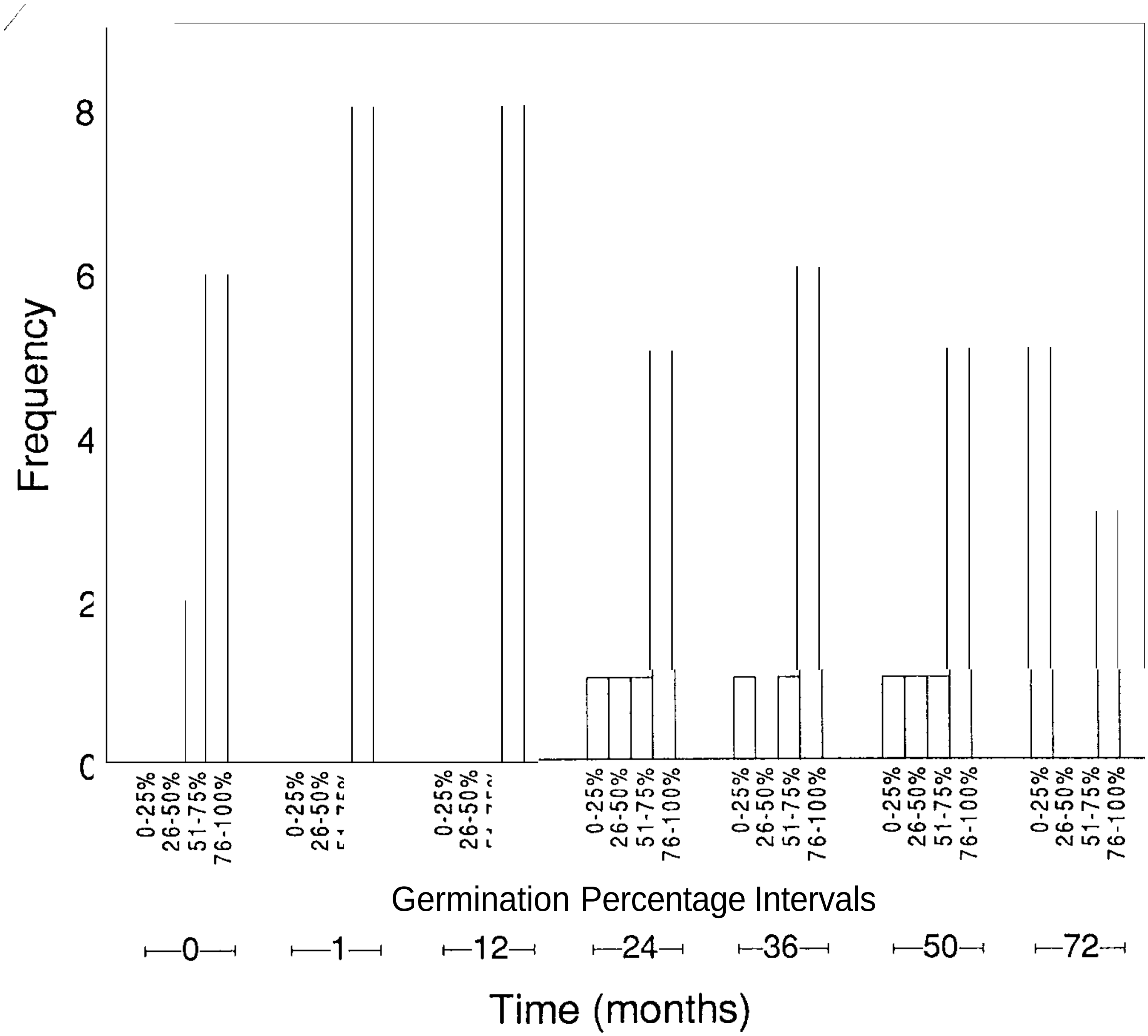

Figure 1. **Frequency of germination percentage intervals** of **buried** yellow **starthistle achenes** over **time** (1983 study).

nonpappus-bearing achenes had declined from 100% at harvest to 73.9% and 81.3%, respectively. By comparison, more than 99% of dry-stored achenes remained viable.

For both dry-stored and buried achenes, there was a significant increase in germination percentages of both achene types from time of collection to one month post-harvest. After one month of burial or storage in the laboratory, germination percentages of pappus-bearing achenes increased from 74.9% to 98.8% and 99.3%, respectively. There was an increase in germinability of the nonpappus-bearing achenes from 58.3% at harvest to 96.8% after one month of burial and 96.5% after one month of dry storage. Levene's test, used to assess the homogeneity of variance in the germinability of buried achenes, indicated that no significant heterogeneity of error variance could be found after 12 months, but after 24 months of burial a non-constant variance was detected ($p < .003$). Regression analysis indicated that the variability in total live achenes (with angular transformation) showed a significant linear increase over 24 months of burial ($r^2 = .98$), $p < .01$). The relationship between time and variability in total live achenes is given by:

$$V = -0.003 + 0.004M.$$

Fate of pappus-bearing achenes buried at various depths.

<u>**1984 study**</u>. There was a significant curvilinear increase in the percentages of pappus-bearing achenes buried at four depths for 13 months. The influence of depth on the percentage of germinable achenes is given by:

$$G = -0.16 + 0.36D - 0.03D^2,$$

where G = germination percentage of achenes, and D = depth of burial ($p < .0001$, $r^2 = .87$). Only 0.5% and 3.9% of achenes at 0.5- and 1-cm depths, respectively, germinated after 13 months of burial. However, 63.1% and

88.1% of achenes at 2.5- and 5-cm depths, respectively, germinated after the same amount of time.

1988 study. The percentages of germinable pappus-bearing achenes after 12 months of burial showed a negative exponential increase with depth (Fig. 2). There were virtually no differences detected between germinable and total live achenes. Maximum survival occurred at depths of 5-om or more, after 12 months.

Packets buried nearest the soil surface (0.5-cm depth) contained a large number of split testa. The influence of depth on the number of split testa is given by the equation:

$$S = 0.069 - 0.760D + 0.044D^2$$

where $S = \log_e$ (number of split testa), and D = depth of burial ($p < .0004$, $r^2 = .98$).

Achene depletion. Arithmetic plots of the number of total live yellow star-thistle achenes and seedlings per square meter showed a curvilinear decline over time for both variables (Fig. 3A). Both the density of achenes in soil and emerged seedlings declined rapidly, to 16.6% and 19.9% of initial abundance, respectively, recorded after 12 months.

There were significant linear and quadratic effects of time on log-transformed propagule density ($r^2 = .999$, $p = .0026$) and significant linear effects of time on log-transformed seedling density ($r^2 = .997$, $p = .0012$) (Fig. 3B). Regression analysis indicated there was a significant interaction between life stage category (In of achenes in soil or In of emerged

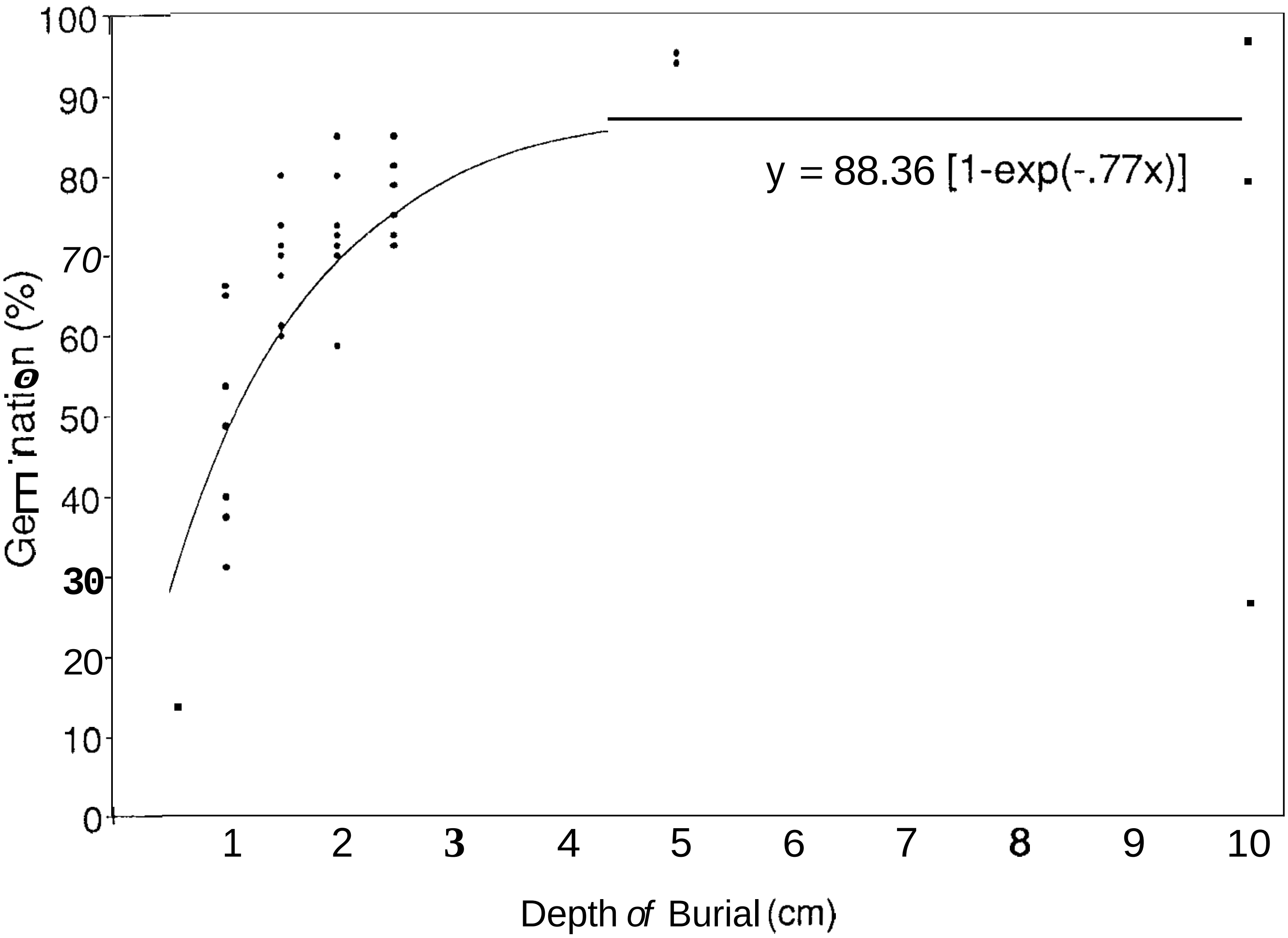

Figure 2. The relationship between germination percentages of yellow star-thistle pappus-bearing achenes and depth of burial after 12 months (1988 study). Solid circles represent actual germination percentages of achenes buried.

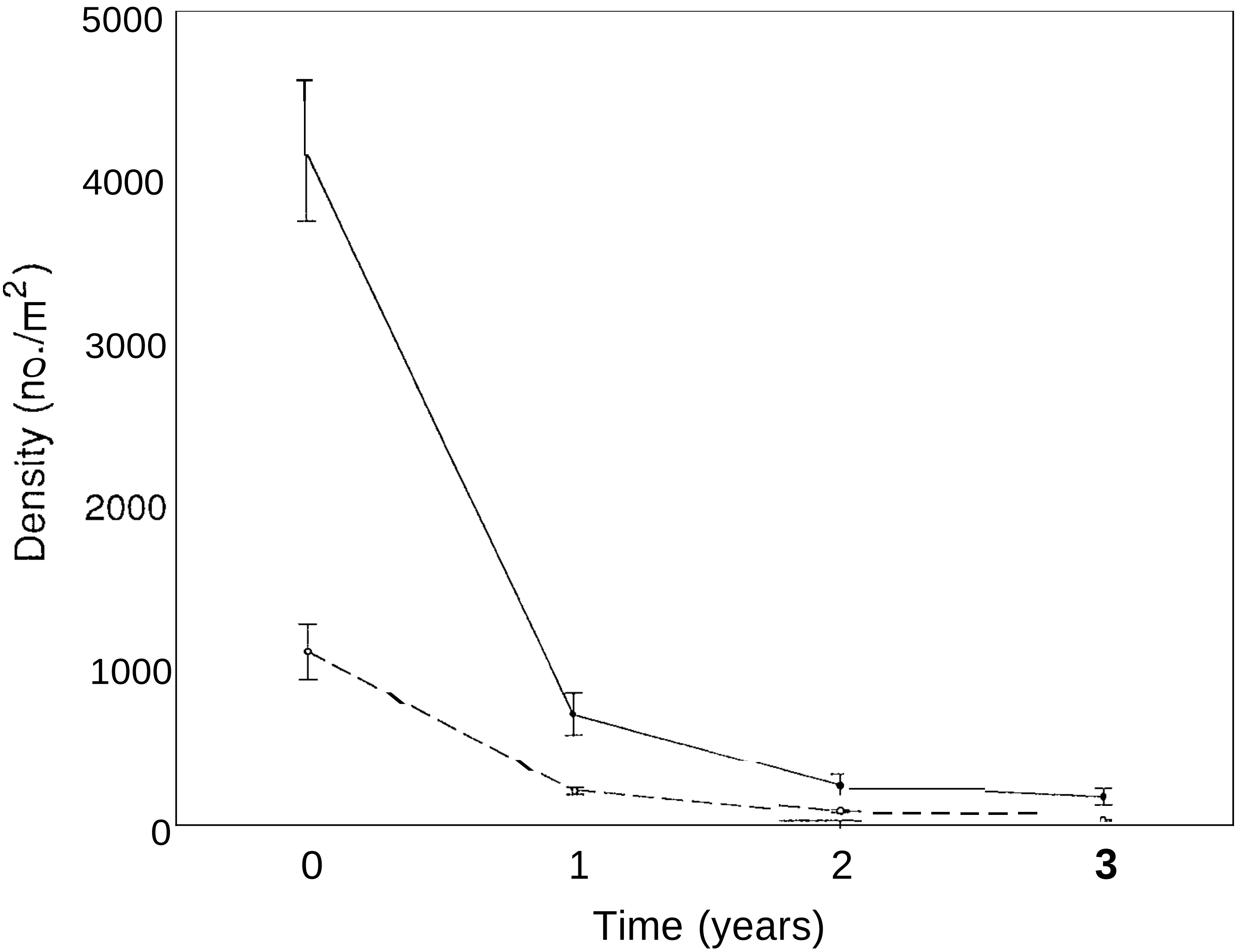

Figure 3A. Density (no./m^2) of total live (germinable plus viable) yellow starthistle achenes recovered from soil (solid lines) and emerged seedlings (hatched lines) per year in the depletion study site: A) arithmetic plot (standard errors are indicated by vertical lines).

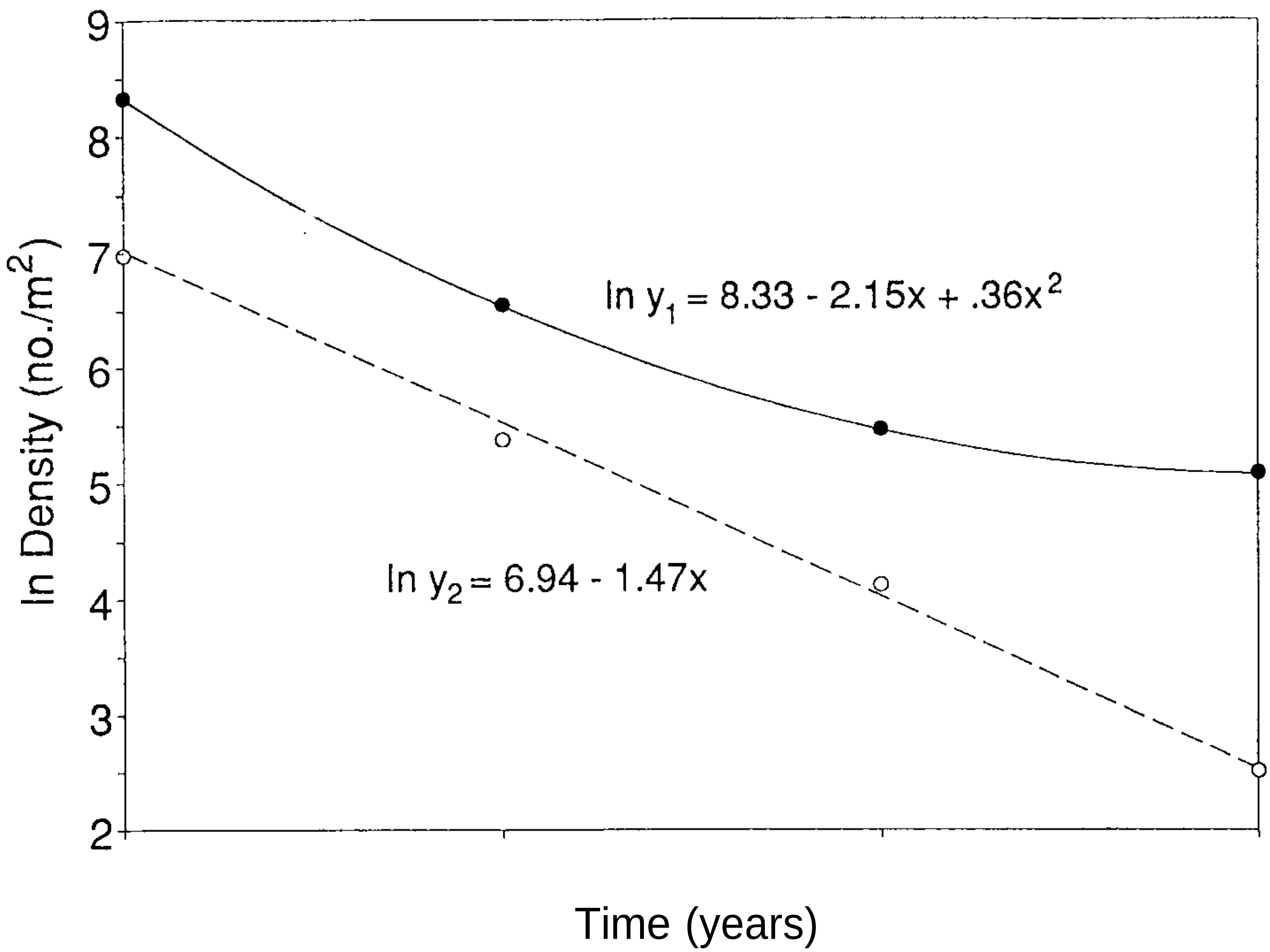

Figure 3B. Density (no./m^2) of total live (germinable plus viable) yellow starthistle achenes recovered from soil (solid lines) and emerged seedlings (hatched lines) per year in the depletion study site: B) semilogarithmic transformation.

seedlings) and the linear and quadratic time effects. Although both achene and seedling densities declined significantly over time, the nature of those reductions was different; decline of achenes in soil was curvilinear while that of emerged seedlings was linear.

Despite the difference in achene and seedling decline, the number of achenes in the upper 2.5-cm of soil appeared to be a good predictor of seedling emergence after autumn rains. The regression relationship between achene density per year and seedling density per year is given by the equation : $X = 2.86 + .74Y$; where X = seeds and y = seedling emergence ($r^2 = .919$, $p = .041$).

DISCUSSION

Although there was a considerable decline of yellow starthistle achenes in some of the buried packets in the 1983 burial study, the large numbers of germinable achenes which remained in other packets after 72 months may, with appropriate disturbance, have been sufficient to support recolonization. At the end of 72 months, the frequency of germination percentage intervals showed two distinct peaks at the extremes (Fig. 1). We believe that this arose primarily from differences existing in the soil environment of the microsites where the packets were buried rather than inherent differences among the replicate achene samples themselves.

The significant decline (after 24 months) in percentages of total live (germinable plus viable) achenes remaining in packets in the 1984 burial study was likely due to germination or death of achenes. Evidence of achene depredation by insects or animals was not observed in any of the burial studies, although it can occur in natural soil seed banks. In any case, there was a consistent pattern whereby variability in germinability or viability of buried achenes increased in a linear fashion for both 1983 and 1984 studies.

The reason(s) for the increases in the germination percentages of buried and dry-stored achenes in the 1984 burial study are not known. However, after-ripening may serve to prevent premature germination of winter annuals, such as yellow starthistle, in dry habitats (3).

The differential loss of germinable pappus-bearing achenes buried at various depths (1984 and 1988 studies) indicated a strong response to burial depth. The nearly complete loss of germinable achenes from shallow depths (0.5- and 1-cm depths, 1984 study; 0.5-cm depth, 1988 study) was felt to be primarily due to germination during burial. Germination was indicated by the typical splitting of testae along the longitudinal axes of the achenes inside the packets in the 1988 study. By contrast, testae of achenes that died during burial were soft but still intact. The abrupt increase in germination percentages of achenes from the 1- to 2.5-cm depths (1984 study), or 0.5- to 1-cm depths (1988 study) suggested that the dynamics of dormancy in this zone changed dramatically. Light may have been an important factor influencing relative dormancy in yellow starthistle achenes in this burial environment. However, other factors could have been important also. For example, diurnally fluctuating temperature (11), soil nitrate (12), and volatile inhibitors arising from metabolism in seeds (4) have been reported to affect the germination response of buried seeds in other plant species. Differences in survival (at the 1-cm depth) between the two studies may have been amplified by variations in exposure to rainfall. In the 1988 study, achenes were exposed to a single wet season (October 1988 to September 1989) whereas, in the 1984 study, achenes received additional rainfall toward the end of the study (October 1984 to November 1985).

In the depletion study, seedling emergence and achene mortality were both major factors responsible for the decline of achenes in soil (Figure 1). In view of the increased survival of pappus-bearing achenes with increases in burial depth, the loss of the major proportion of achenes in the study site during the first year was most likely due to the distribution of achenes on or near the soil surface. Yadav and Tripathi (13) demonstrated that seeds of three Eupatorium spp. remain near the surface in undisturbed soil. Likewise, most achenes of yellow starthistle, especially those dispersed from plants during the initial year of the study (1983), probably remained near the surface as well since soil and duff layers were relatively undisturbed,

Although there was a sharp decline in seedling emergence and achene recovery in the second and third years of the study, the soil seed bank was obviously buffered against total depletion in a single year. Thus, under the conditions of the study in which achene rain was prevented, there was an exponential rate of achene loss from the emergence zone. Consequently, dormant achenes in the lower strata became the major constituent in the soil profile. Conversely, under conditions of natural achene rain, a small proportion of achenes are displaced downward in the soil profile by natural factors and enter a dormant state. Over time these achenes may accumulate to form a persistent seed bank that has the potential for recolonization.

LITERATURE CITED

1. Anon. 1980. Soil Survey of Placer County, California, (Western Part). USDA Soil Conserv. Serv. in Coop. with Univ. California Exp. Stn. 204 pp.

2. Callihan, R. H., R. L. Sheley, and D. C. Thill. 1982. Yellow Starthistle identification and control. Univ. of Idaho Curr. Inf. Ser. No. 634. 4pp.

3. Grime, J. P., G. Mason, A. V. Curtis, J. Rodman, S. R. Band, M. A. G. Mowforth, A. M. Neal, and S. Shaw. 1981. A comparative study of germination characteristics in a local flora. J. Ecol. 69:1017-1059.

4. Holm, R. E. 1972. Volatile metabolites controlling germination in buried weed seeds. Plant Physiol. 50:293-297.

5. Maddox, D. M. 1981. Introduction, phenology, and density of yellow starthistle in coastal, intercoastal, and central valley situations in California. U.S. Dep. Agric. Res. Serv., Agric. Res. Results, ARR-W-20/July. 33 pp.

6. Maddox, D. M. and A. Mayfield. 1985. Yellow starthistle infestations are on the increase. California Agric. 39:10-12.

7. Maddox, D. M., A. Mayfield, and N. Poritz. 1985. Distribution of yellow starthistle (Centaurea solstitialis) and Russian knapweed (Centaurea repens). Weed Sci. 33:315- 327.

8. SAS User's Guide:Statistics. Version 5 ed. 1985. SAS Inst. Inc., Cary, NC.

9. Snedecor, G. W. and W. G. Cochran. Statistical Methods. 7th ed. Ames, IA: Iowa State Univ. Press; 1980:253-254.

10. Sokal, R. R. and F. J. Rohlf. Biometry. 2nd ed. New York: W. H. Freeman and Co, 1981:427-428.

11. Thompson, K. and J. P. Grime. 1983. A comparative study of germination responses to diurnally-fluctuating temperatures. J. Appl. Ecol. 20:141-156.

12. Vincent, E. M. and E. H. Roberts. 1977. The interaction of light, nitrate and alternating temperature in promoting the germination of dormant seeds of common weed species. Seed Sci. & Technol. 5:659-670.

13. Yadav, A. S. and R. S. Tripathi. 1982. A study on seed population dynamics of three weedy species of Eupatorium. Weed Res. 22:69-76.